Biological Science

12A/2004G391

Distribution of light elements including Ca and P in mammalian cells calculated using soft X-ray images obtained by contact microscopy

Atsushi ITO*[1], Yohei OHNO[1], Takahiro ARIKAWA[1], Kunio SHINOHARA[2]
[1]School of Engineering, Tokai Univ., Hiratsuka, Kanagawa 259-1292, Japan
[2]JASRI/SPring-8, Sayo-gun, Hyogo 679-5198, Japan

Introduction

Soft X-ray microscopy has a unique characteristic to obtain high-resolution maps of light elements that constitute biological specimens. In addition to major C, O and N elements, distribution of minor light elements such as Ca and P provides useful information about their participation in cellular physiological responses.

For the quantitative mapping of these elements, we have been developing computer programs to calculate light elements from a set of soft X-ray images taken at wavelengths including both sides across absorption edges of contained elements. Contact X-ray microscopy in combination with an electronic zooming tube is a useful tool for this purpose in the applicability to the wide wavelength region to cover absorption edges of intracellular light elements. In the computer algorithm, subtraction of absorption between both sides of the absorption edges is performed for each pixel to obtain the map of elemental contents. An iteration procedure is also introduced to find absorption changes at the absorption edges that are hidden by a large absorption of major light elements [1]. In our previous algorithm a set of the above subtracted equations is solved simultaneously. However this algorithm required a lot of calculation time.

In the present study, we have improved the algorithm to reduce the calculation time drastically. The calculated results gave the same values as those obtained with the previous one.

Materials and Methods

Contact X-ray microscopy with an electronic zooming tube was used to obtain X-ray images of dried human cancer HeLa cells in the wavelength range from 1.5 nm to 10 nm at the BL-12A beamline. The computer algorithm to calculate elemental maps is as follows:

The absorption (A) for each pixel is expressed as shown in the Eq. (1).

$$A = \mu_C \rho_C x + \mu_O \rho_O x + \mu_N \rho_N x + \mu_P \rho_P x + \cdots \qquad (1)$$

where μ_c etc. are mass absorption coefficient of carbon etc., and $\rho_c x$ etc. denote mass thickness of carbon etc. Step 1: Suppose that in the absorption spectrum an absorption jump at the absorption edge is apparent for carbon. Then the following subtraction equation is obtained:

$$A_C = A_{CS} - A_{CL} = (\mu_{C-CS} - \mu_{C-CL})\rho_C x \qquad (2)$$

where CS is a wavelength of the shorter side of the C-K edge, and CL is a wavelength of the longer side of the C-K edge. $\mu_{C\text{-}CS}$ and $\mu_{C\text{-}CL}$ show mass absorption coefficients of C at the wavelength of CS and CL. The first approximated value of carbon content ($\rho_c x$) was obtained from Eq. (2).

Step 2: $A\text{-}\mu_C\rho_C x$ is calculated to detect absorption jumps besides carbon. If we find an absorption jump at the oxygen absorption edge, another equation is described in addition to the carbon case. The two equations are solved, and the second approximated content of carbon is the sum of the first approximated value and the value from the second equation. The first approximated value of oxygen is also calculated.

Then the steps of 1 and 2 are repeated until no absorption jumps are detected.

Results and Discussion

Fig. 1 shows the distributions of phosphorus and calcium in HeLa cells. The results show the predominant distribution of phosphorus in the nuclei, and interestingly calcium was also present preferentially in the nuclear region. The distribution and the elemental content were identical with those in our previous study. Compared with our previous algorithm, the calculation time was largely reduced to one fifth.

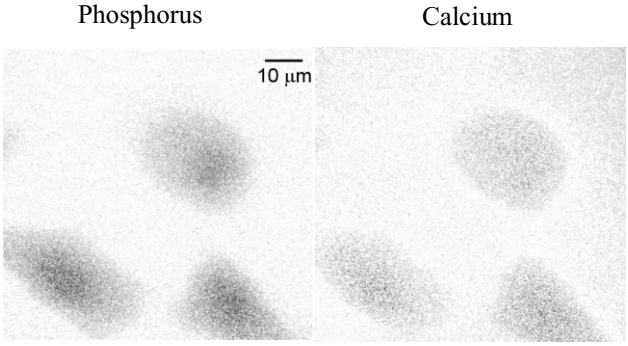

Fig. 1. Distributions of phosphorus and calcium in HeLa cells.

References

[1] Ito A., Matsuda H., Kitajima Y. and Shinohara K., *J. Phys. IV France*, **104** (2003) 297-300

*aeito@keyaki.cc.u-tokai.ac.jp